DEPARTMENT OF HEALTH AND HUMAN SERVICES

NOTE TO THE FILE

JUL 14 1995

Subject: Bt-Resistant Corn

Keywords:

Corn, *Bacillus thuringiensis* subsp. *kurstaki*, *cryIA(b)*, Bt-toxin, Insect Resistance, Pesticide, *Streptomyces hygroscopicus*, Phosphinothricin Acetyltransferase (PAT, *bar*),

Background

On January 14, 1993, representatives of Ciba-Geigy met with FDA to discuss their development of European corn borer-resistant corn expressing the δ-endotoxin of *Bacillus thuringiensis*. In a submission dated March 2, 1995, Ciba-Geigy provided summary information to support their safety and nutritional assessment of their new corn line Event 176. On May 30, 1995, Ciba-Geigy supplemented their submission with information discussed in a telephone conference held May 12, 1995. In a telephone conference held June 16, 1995, Ciba-Geigy indicated that they do not intend to use their Event 176 Corn line, or corn lines developed through cross hybridizations with corn lines, as silage sources for animal feed uses.

Intended Effect and Food/Feed Use

The intended technical effect of this genetic modification of corn plants is to confer resistance to lepidopteran pests. Corn grain (kernels) are primarily used for animal feed and human food. The foliar parts of corn plants are primarily used for the production of silage which is used in animal feed. Corn oil, corn syrup, and cornmeal are the primary by-products of corn grain that are used in human foodstuffs. Corn oil is commonly used as a vegetable oil in human food. Corn syrup is used primarily as a sweetener in human food. Corn by-products used in animal feed include: corn gluten feed and meal; corn germ meal; and hominy feed.

Event 176 Corn has been modified to express the *cryIA(b)* gene from *B. thuringiensis* subsp. *kurstaki* (B.t.k.) which encodes a protein (Bt-toxin, CRYIA(b) protein) that is toxic to certain lepidopteran insect pests. Ciba-Geigy states that expression of the *cryIA(b)* gene in corn plants results in increased resistance to lepidopteran insect pests. The toxicity of the CRYIA(b) protein is reported to be very specific to lepidopterans.

Molecular Alterations and Characterization

According to Ciba-Geigy, the inserted DNA is capable of expressing two proteins: 1) CRYIA(b); and 2) phosphinothricin acetyltransferase (PAT). The safe use of Bt-toxins as pesticides and the use of selectable markers as pesticidal inert ingredients in the development of pesticide resistant plant varieties are regulated by the Environmental Protection Agency (EPA) under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA). Therefore, we have not addressed the safe use of Bt-toxin as a pesticide or the safe use of PAT as a pesticidal inert ingredient.

Ciba-Geigy provided a summary of information they have obtained pertaining to the expression of the CRYIA(b) and PAT proteins in their Event 176 Corn.

Compositional Analysis

According to Ciba-Geigy, the only endogenous toxin produced in corn plants is the hydroxamic acid of 2,4-Dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (DIMBOA). Ciba-Geigy reported that DIMBOA levels are highest in young corn plants, DIMBOA is absent in corn kernels, and DIMBOA is highly unstable. Ciba-Geigy examined the levels of DIMBOA in leaf tissue from Event 176 Corn plants and non-transformed control plants. They reported that they observed no statistically significant difference in leaf tissue DIMBOA levels between Event 176 Corn plants and non-transformed control corn plants.

Nutritional Assessment

Grain

Based on the nature of the genetic modification, it is expected that Event 176 Corn would not materially differ in composition from other corn varieties. To confirm this expectation, Ciba-Geigy analyzed the nutrient composition of grain obtained from Event 176 Corn and a parental control line. The composition of grain derived from Event 176 Corn and non-transformed control plants were analyzed for: total ash, fat, crude fiber, moisture, protein, starch, carotenoids (xanthophylls and ß-carotene), amino acid composition, and fatty acid profiles. No statistically significant differences were reported by Ciba-Geigy in the levels of ash, crude fiber, xanthophyll, ß-carotene, or major fatty acid content, between grain derived from Event 176 Corn and non-transformed control plants. Ciba-Geigy reported that they did observe some sporadic differences in total grain protein, starch, and fat content between some Event 176 Corn genotypes and their controls, but these differences were minimal, and within the established range for corn. Ciba-Geigy considers these

differences to be biologically and nutritionally insignificant. Ciba-Geigy also reported observing statistically significant differences in the relative proportion of one or more amino acids in the kernels of all Event 176 Corn genotypes and their control plants. These differences were reportedly small and did not alter the overall amino acid profile. From their nutritional composition analysis, Ciba-Geigy concludes that the composition of Event 176 Corn grain is indistinguishable from non-transformed counterparts.

Vegetative Tissues

During the May 12, 1995, teleconference, CVM inquired about the firm's assessment of the nutritional value of silage derived from Event 176 corn used in animal feed. CVM felt that the assessment would be strengthened by a proximate analysis of several nutritional components (e.g., fat, ash, acid detergent fiber (ADF), neutral detergent fiber (NDF), moisture, calcium, and phosphorous) of the vegetative tissue. Alternatively, analysis of agronomic characteristics that correlate with nutritional components may suffice.

In their submission dated May 30, 1995, Ciba-Geigy submitted additional information on the nutritional value of vegetative tissue derived from Event 176 Corn.

Ciba-Geigy determined the total protein per gram dry weight as measured by total nitrogen content of leaf and stalk tissues. No significant difference in vegetative tissue nitrogen levels were reported between Event 176 Corn and the parental control.

Ciba-Geigy did not report proximate analysis of other nutritionally important components (e.g., fat, ash, ADF, NDF, moisture, calcium, phosphorous, etc.) of the vegetative tissues used for silage from Event 176 Corn. However, Ciba-Geigy did report assessing several agronomic characteristics, which in their view would reflect any alterations in the nutritional quality of silage produced from Event 176 Corn. According to Ciba-Geigy: "[t]he overwhelming assessment of the agronomists and breeders was that except for tolerance to ECB [European Corn Borer], the performance of the Bt corn hybrids is indistinguishable from their non-transformed isogenic counterparts."

Ciba-Geigy concluded from these studies that: "[t]he lack of measurable phenotypic variance between Ciba Seeds' Bt Corn and nontransgenic corn in the traits described indicates no unintended effects resulting from the transformation process or expression of the inserted genes."

However, CVM continues to believe that the assessment of the nutritional value of silage derived from Event 176 corn would be strengthened by analysis of other nutritional components ADF, NDF, moisture, ash, calcium, and phosphorus of vegetative tissues.

Wholesomeness Studies

Ciba-Geigy reported that they have conducted acute oral toxicity studies in mice and bobwhite quail, using an enriched Event 176 Corn leaf protein preparation containing truncated CRYIA(b) protein, and an acute oral toxicity study in mice using native CRYIA(b) protein. According to Ciba-Geigy, the results indicated no adverse effects resulting from the CRYIA(b) treatment.

Conclusions

Ciba-Geigy has concluded that no issues of concern regarding the food or feed safety were raised by their analysis of their Event 176 Corn. At this time, based on Ciba-Geigy's description of its data and analyses, the Agency considers Ciba-Geigy's consultation on corn grain (kernels) from their Event 176 Corn line to be complete.

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